The Use of HSP70 for Prevention of Consequences of Unavoidable Stress in Rats

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We studied whether intranasal treatment with HSP70 can be used for the correction of maladaptive behavior in rats after unavoidable stress. After 3 intranasal injection of HSP70 in a dose of 3.25 μg we observed normalization of adaptive behavior after unavoidable stress and improvement of physiological reserves of the organism.

Key Words: *heat-shock proteins; unavoidable stress; behavior; protein peroxidation*

Study of the mechanisms protecting the organism from stress is an urgent problem of modern physiology and clinical medicine. The needs for these investigations are determined by various social factors, including increased information flow, emotional load, and threat of terrorism. These factors negatively affect the systems maintaining homeostasis in humans.

In light of this, new pharmacological preparations improving adaptive capacities of the organism attracted much recent attention. Endogenous adaptogens are of particular interest in this respect. Heat-shock proteins (HSP) are potent endogenous molecules producing general protective and cytoprotective effects [1,4,5]. Various factors can induce synthesis of HSP, which confirms universal nature of this mechanism of cell response to extreme factors [8]. HSP synthesis is primarily induced by oxidized proteins, whose amount considerably increases during stress [11]. The family of HSP70 was extensively studied. These proteins participate in cell reparation and provide biosynthesis and structural integrity of proteins in abnormal cells [4]. HSP70 *in vivo* protect neurons from the influence of adverse factors that cause their elimination in various brain structures [5,7].

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In vivo studies of the protective effect of HSP in animal models of human mental disorders hold much promise for the development of new approaches to the correction of various disturbances induced by stress or resulting from neurodegenerative processes.

One of the major problems limiting the use of HSP in clinical practice is their transport to brain cells. Intranasal administration of HSP holds promise in this respect. Olfactory epithelial cells have numerous connections with central olfactory structures. The olfactory tract transmits chemical signals to various brain regions, including the neocortex, striatum, amygdala, and preoptic hypothalamus. These structures play a key role in the regulation of homeostasis and formation of adaptive behavior [7,8].

We studied the protective effect of HSP70 administered intranasally to rats subjected to unavoidable stress. This stress causes the phenomenon of learned helplessness in rats and serves as a model of depression in humans. Since depressive syndrome includes severe behavioral disorders and disturbances in locomotor and exploratory activities, the influence of HSP70 on behavioral characteristics of rats was studied in the open-field test.

Stress factors and neurodegenerative diseases intensify free radical processes in the brain [6]. Oxidative modification of plasma proteins most adequately reflects the directionality of free radical processes in the organism. Spontaneous protein peroxidation

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(PPO) proceeds without stimulators of oxidative modification. Induced PPO is initiated by Fenton's reagent containing Fe^{2+} and H_2O_2. This system is characterized by pronounced oxidative modification. The intensity of spontaneous PPO reflects the general physical state, while induced PPO demonstrates potential capacities of the organism [3]. In the present study we evaluated the influence of intranasal treatment with HSP70 on PPO in the plasma from rats subjected to unavoidable stress.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 170-220 g. To induce the phenomenon of learned helplessness the animals were subjected to unavoidable stress: electrical stimulation (1 mA, 50 Hz) for 15 sec with 45-sec intervals. The rats were placed in a chamber (130×16×13 cm) with electrified floor. Each animal received 60 electric shocks. Control rats maintained in similar chambers for 1 h were not subjected to electrical stimulation.

The state of learned helplessness after unavoidable shock persists for at least 6 days. Stressed rats were divided into 2 subgroups. Stressed animals of subgroup 1 intranasally received HSP70 in a daily dose of 3.25 μg in physiological saline (5 μl into each nostril) immediately and 2 days after electrical stimulation. The preparation of HSP70 was obtained as described elsewhere [12]. Stressed rats of subgroup 2 intranasally received an equivalent volume of bovine serum albumin (BSA) in the same concentration. Control animals not subjected to unavoidable shock received similar treatment. Behavioral characteristics of control and stressed rats were studied in the open-field test 5 min after the last intranasal administration of preparations. The open field was a rectangular area (100×100×45 cm). The floor was divided into squares

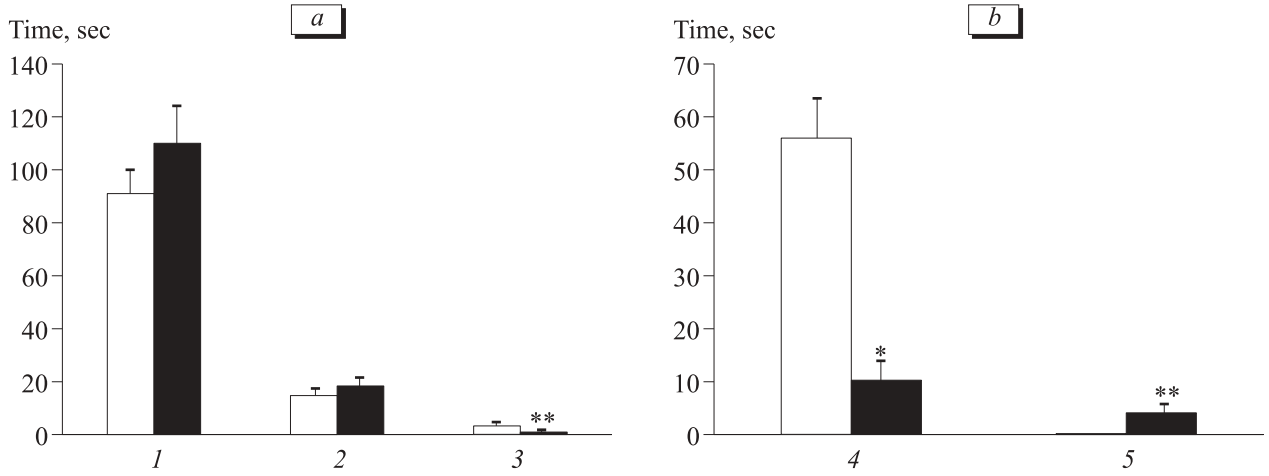

Fig. 1. Effect of intranasal treatment with HSP70 on open-field behavior of intact rats. Light bars: control rats (n=12). Dark bars: treated rats (n=15). Here and in Fig. 2: *a*) number of crossed squares (*1*) and rearing postures (*2*) and defecation rate (*3*); *b*) period of freezing behavior (*4*) and time spent in the center of the open field (*5*). Here and in Fig. 2: *p<0.01 and **p<0.05 compared to the control.

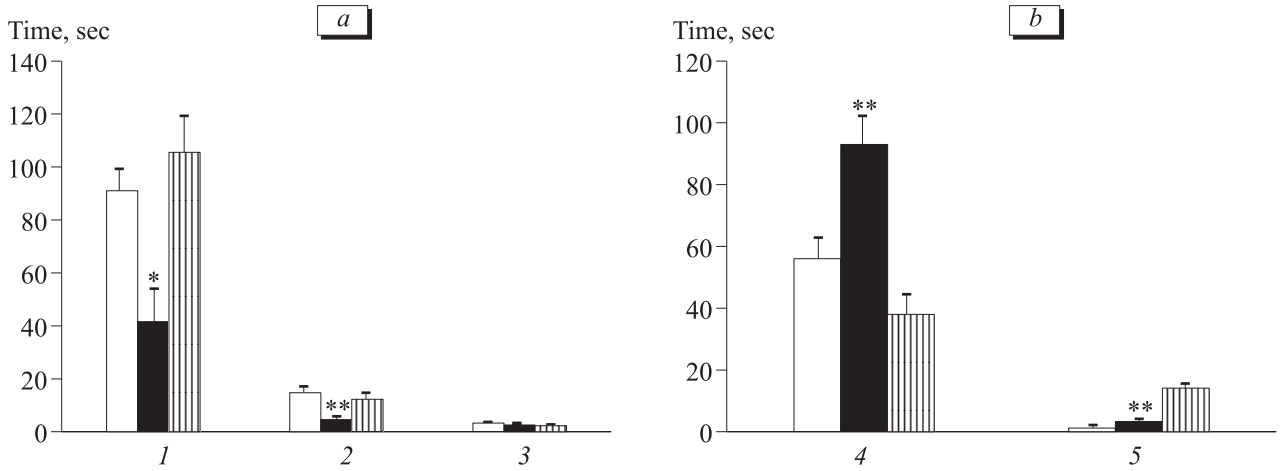

Fig. 2. Open-field behavior of rats subjected to unavoidable shock. Light bars: control rats (n=10). Dark bars: rats receiving BSA (n=12). Shaded bars: rats receiving HSP70 (n=12).

TABLE 1. PPO in the Plasma from Rats Subjected to Unavoidable Shock and Intranasally Receiving HSP70 ($M \pm m$, n=8)

PPO	Group	
	BSA	HSP70
Spontaneous PPO		
270 nm	0.082±0.022	0.085±0.01
363 nm	0.144±0.012	0.165±0.01
370 nm	0.163±0.01	0.168±0.1
Induced PPO		
270 nm	0.132±0.035	0.151±0.023
363 nm	0.101±0.023	0.144±0.021*
370 nm	0.092±0.02	0.149±0.017*

Note. *p<0.05 compared to animals receiving BSA.

(20×20 cm). The area was illuminated with a 60 W lamp positioned at a height of 50 cm from the floor. The rat was placed in the center of the field. The behavior of each rat was studied for 5 min. We recorded the number of crossed squares (horizontal locomotor activity), number of rearings (exploratory activity), and time of freezing. Emotionality of animals was determined by defecation rate. The degree of anxiety was estimated by the time spent in 4 central squares. The effect of HSP70 on behavior of intact rats in the open field was also determined.

The effect of HSP70 on oxidative modification of plasma proteins was studied by the method of R. L. Levin [10] with modifications of E. E. Dubinina *et al*. [2]. The differences between rats of the main group and stressed intact animals were evaluated.

RESULTS

HSP70 had no effect on horizontal and vertical exploratory activity of intact rats. These rats did not differ from animals receiving physiological saline in the number of crossed squares and rearing postures (Fig. 1). However, HSP70 markedly decreased the time of freezing and increased the time spent in the center of the open field. These changes reflect reduction of anxiety. Moreover, we observed a decrease in emotionality of rats treated with HSP70. Probably, the animals receiving HSP70 became more adapted to new environmental conditions.

Two days after unavoidable shock behavioral parameters of rats receiving BSA differed from those of unstressed animals (Fig. 2). Horizontal and vertical activities decreased and the time of freezing increased. The open-field behavior of stressed rats treated with HSP70 did not differ from the control.

Spontaneous PPO in rats receiving HSP70 and BSA did not differ from the control (stressed intact animals, Table 1). It should be emphasized that HSP70 increased the content of products of induced PPO in stressed rats by 40-60% (363 and 370 nm, respectively). Intensification of induced PPO reflects the increase in adaptive capacities of the organism, which improves the resistance to extreme factors.

Our results indicate that intranasal administration of HSP70 can be used for evaluation of adaptive mechanisms and for correction of behavioral reactions.

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